16707

Incidence of Mammary Tumors in Castrate and Non-Castrate Male Mice Bearing Ovarian Grafts.*

ROBERT A. HUSEBY AND JOHN J. BITTNER.

From the Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.

Although extensive investigations have been carried out determining the state of function of ovaries grafted to castrate, non-castrate and cryptorchid male animals (for review articles see [1,2,3]), there appear to be only 2 reports of the successful production of mammary carcinoma in male mice by this surgical procedure. Murray[4] observed mammary tumors in 7.1% of 210 dba castrate male mice bearing intraperitoneal ovarian grafts while de Jongh and Korteweg[5] report the development of mammary carcinoma in 9 of 16 male mice similarly treated. Since it has been subsequently established that the rodent liver very effectively inactivates estrogenic substances carried through the portal circulation[6,7] and that after castration the adrenals of male mice of at least one strain may produce sufficient estrogen for the development of mammary cancer,[8] it was felt worth while to reinvestigate this problem. The data presented here represent a completion and extension of those previously reported in preliminary form.[9]

Materials and Methods. All mice used in these experiments were F_1 hybrids between the A and Z (C_3H) stocks maintained in this laboratory. As virgin female mice of the reciprocal A $\times$ Z cross exhibit a different incidence of mammary carcinoma as well as a different average age of tumor development,[10] approximately equal numbers of animals of the two crosses were employed in each of the groups in these experiments. All surgical procedures were carried out soon after weaning, *i.e.*, when the mice were 4 to 6 weeks of age, after which the animals were housed in wooden boxes, fed Purina Fox Chow *ad libitum*, and inspected once a week for the appearance of subcutaneous tumors. All such tumor masses were studied microscopically.

The method of ovarian transplantation was the same as that previously employed in female mice,[11] consisting of the removal of the ovaries from their encompassing ovarian sac and inserting them deep into the tissue of the axilla through a small skin incision. In our hands this method has proven superior to intraperitoneal implantation in mice since aseptic technics need not be employed as is necessary for intraperitoneal implantation, and the danger of portal vascularization is obviated. From the data obtained with ovarian transplantation into ovariectomized female mice, it would appear that ovaries grafted in this manner function very similar to those remaining in place within the abdomen. Castration was carried out via a transcrotal approach and was done at the

* Assisted by grants from the Minnesota Division of the American Cancer Society, the Citizens Aid Society of Minneapolis, The Graduate School Cancer Research Fund of the University of Minnesota, and the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

1 Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

2 Smelser, G. K., *Physiol. Zool.*, 1933, **6**, 396.

3 Gardner, W. U., *Endo.*, 1935, **19**, 656.

4 Murray, W. S., *J. Cancer Res.*, 1928, **12**, 18.

5 de Jough, S. E., and Korteweg, R., *Acta brev. Neerland.*, 1935, **5**, 126.

6 Zondek, B., *Skandinav. Arch. F. Physiol.*, 1934, **70**, 133.

7 Golden, J. B., and Severinghaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 361.

8 Wooley, G., Fekete, E., and Little, C. C., *Endo.*, 1941, **28**, 341.

9 Huseby, R. A., Smith, F. W., and Bittner, J. J., *Cancer Research*, 1946, **6**, 494.

10 Bittner, J. J., and Huseby, R. A., *Cancer Research*, 1946, **6**, 235.

11 Huseby, R. A., and Bittner, J. J., Proc. Fourth Inter. Cancer Res. Congress, 1947, in press.

TABLE I.
Summary of Tumor Data from Castrate and Non-castrate Hybrid Males Transplanted with Ovaries. For comparison similar data is given for intact female and ovariectomized ovarian transplanted females of the same hybrid cross.

Group	No. animals/ No. cancerous	% cancerous	Avg age: Of cancer development, mo.	Avg age: Non-cancerous deaths, mo.
Mammary tumors in F_1 hybrid males bearing transplanted F_1 hybrid ovaries.				
Castrate	31/27	87.1	11.7	16.9
Non-castrate	39/1	2.5	19.4	27.0

Mammary tumors in normal female mice of the A × Z reciprocal crosses.[10]

Stock	Virgin: % cancerous	Virgin: Avg age of cancer, mo.	Breeding: % cancerous	Breeding: Avg age of cancer, mo.
AZF_1	92.5	15.2	98.0	9.7
ZAF_1	73.2	19.1	97.6	10.1
Average	82.9	16.9	97.8	9.9

Mammary tumors in ovariectomized female mice of the A × Z crosses bearing transplanted hybrid ovaries.[11]

Stock	% cancerous	Avg age of cancer, mo
AZF_1	92.3	15.9
ZAF_1	48.1	21.1

same time as the ovarian transplantation.

Results. Data dealing with mammary tumor development are summarized in Table I. From these it is evident that the presence of the normal testes profoundly affects the development of mammary cancer in male mice bearing ovarian grafts. It is evident, further, that castrate male mice with ovaries develop carcinomas of the mammary gland at a significantly earlier age than do normal virgin females or ovariectomized females bearing ovarian grafts. In fact, in this respect, these castrate male mice bearing ovaries more closely resemble breeding females of this hybrid cross than they do virgin females. This was further emphasized by the fact that there was no difference in the male mice of the two reciprocal crosses as far as either the incidence of tumors or the average age at which these tumors developed.

The ovaries of all the castrate animals that developed mammary tumors and of 12 non-castrate males sacrificed between 362 and 501 days of age were studied employing routine histological procedures. To demonstrate finer cytological details, 2-month-old ovarian grafts carried in castrate and non-castrate males and in ovariectomized female mice were fixed in resorcinol-formol,[12] sectioned at 2 micra, and stained according to the Altmann-Bensley method.

The appearance of the grafts in ovariectomized females was essentially that of normal ovaries. Those recovered from non-castrate males differed mainly in that corpora lutea were absent, and thus the size of these transplants was smaller than that of the transplants carried in ovariectomized females. In the grafts of all mice of this group that were studied, however, numerous normal appearing follicles were present. These ranged in size from primary follicles to ones of moderate size possessing well formed antra. The interstitial cells of these ovaries were somewhat atypical in that with a routine hematoxylin-eosin stain their cytoplasm appeared moderately hypertrophied and more vacuolated than is normal for this strain of mouse. However, the over-all size of the "interstitial organ" was not particularly excessive. In all 20 non-castrate mice sacrificed for histological study, well maintained ovarian

[12] Huseby, R. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 122.

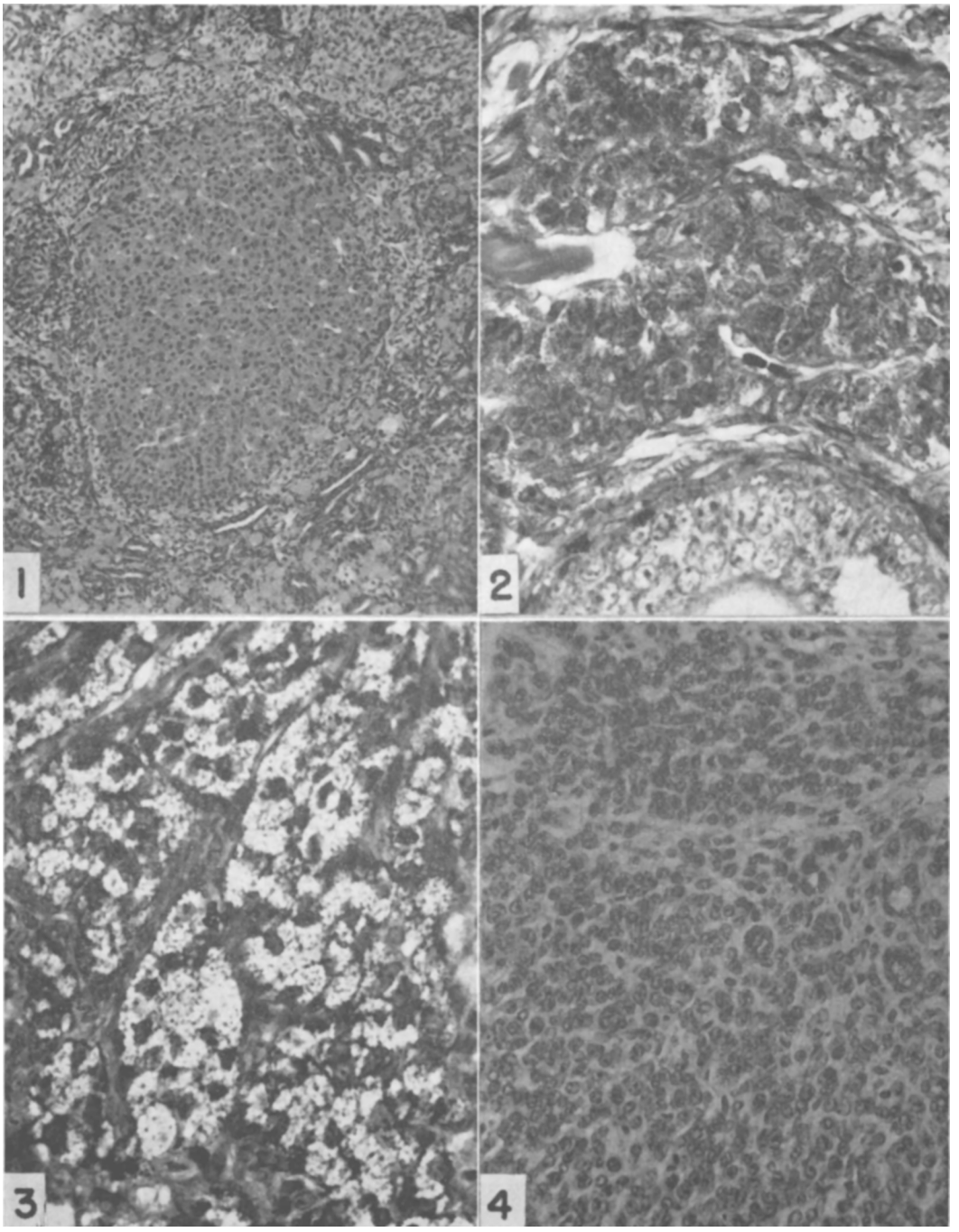

FIG. 1. An area of an ovarian graft carried in a castrate male mouse showing a small corpus luteum. Bouin's fixation and hematoxylin eosin stain. Mag. ×140.

FIG. 2. A group of interstitial cells in an ovarian graft from an ovariectomized female. Note the numerous granules in the cytoplasm and the absence of vacuolization. Resorcinol-formol fixation and Altmann-Bensley stain. Mag. ×800.

FIG. 3. An area of interstitial cells in an ovarian graft carried in a castrate male. Note the extensive vacuolization of the cytoplasm and the paucity of granules. Fixation and staining as in Fig. 2. Mag. ×800.

FIG. 4. An area from a granulosa cell tumor developing in an ovarian graft carried in a non-castrate male. Fixation and staining as in Fig. 1. Mag. ×150.

grafts were found.

The ovarian grafts recovered from castrate male mice differed from those of non-castrate males and of normal females. Although most reports of histological examination of ovarian grafts carried in castrate male animals, mainly in guinea pigs, record a complete absence of corpora lutea, not infrequently in the ovaries of these castrate male mice typical small corpora lutea were encountered (Fig. 1). The ovaries of F_1 hybrid females of the A $\times$ Z cross more closely resemble those of the Z parent in that corpora lutea are retained for a considerable time and thus normally this element makes up a large portion of the ovarian mass during the reproductive period in the animal's life.[11] It is evident, therefore, that although corpora lutea appeared not infrequently in the ovarian grafts of these castrate males, they were by no means as prevalent as in ovaries maintained in female animals. Conversely, however, the interstitial tissue of these transplanted ovaries was greatly hypertrophied and the cytoplasm of the individual interstitial cell possessed many large vacuoles. Follicles were numerous, appeared cytologically normal, but in general more large follicles with medium to large sized antra were evident than is the case in normal ovaries in female mice. In size these ovarian grafts considerably exceeded those of the non-castrate males and equaled or slightly exceeded those in ovariectomized females. Weights were not taken because of the difficulty of dissecting the ovaries completely free of surrounding connective tissue.

The detailed cytological study of the ovarian grafts revealed the follicular elements to be normal in all instances as were the cells of the large corpora lutea in the female grafts and of the small corpora lutea in the grafts of the castrate males. The interstitial cells of the 3 groups of ovaries differed greatly, however. In the female grafts studied, as well as in normal females of this cross, the cytoplasm of the interstitial cells was generally crowded with rather large, brilliantly fucsinophilic granules (Fig. 2). In the non-castrate males some cells possessed a large number of these granules while the cytoplasm of others was rather extensively vacuolated with a loss of granulation. The cytoplasm of the interstitial cells of the ovaries in the castrate males was uniformly hypertrophied and extensively vacuolated with but few fucsinophilic granules remaining (Fig. 3).

These histological studies would suggest that the ovaries in the castrate males were subjected to a greater pituitary stimulation than were those in the non-castrate group.[13] In the majority of cases the adrenals of castrate tumor bearing animals were studied also, and no example of adrenal hyperplasia was noted.

In view of the great difference in the development of mammary carcinoma in the two groups of animals of this experiment, a histological study of their mammary glands was made employing the whole mount technic. The glands of the castrate males were found to be extensively developed with large ducts extending throughout the major portion of the various fat pads. In addition to ductular growth there were many areas of alveolar development, some of which formed typical pre-cancerous lesions while others appeared as more normal lobules in the early stages of secretion, *i.e.*, similar to those seen in normal female glands about the time of parturition. In some animals the mammary ducts were filled with a white, milk-like secretion. As has been observed by others, all 10 glands rarely develop in male mice receiving estrogenic stimulation. As contrasted to this picture, no definite example of mammary gland development was found in the non-castrate males. In some a few fine thread-like ducts could be seen extending a considerable distance into a fat pad, particularly in the number two position, but such development is not infrequent in normal male mice of this hybrid cross. Neither lateral buds nor alveoli were seen in these glands. Examination of the glands of the one non-castrate male that developed a mammary adenocarcinoma showed no more mammary growth than was seen in the other animals of the group.

It seemed possible to test the function of the well preserved ovarian grafts of non-

[13] Pfeiffer, C. A., and Hooker, C. W., *Anat. Rec.*, 1942, **83**, 543.

TABLE II.
Development of Uterine and Vaginal Grafts and Mammary Glands in Mice 2 Months After Ovarian Transplantation.

Ovaries transplanted to	Uterine grafts	Vaginal grafts	Mammary glands
Ovariectomized females	Developed. Avg 6.0 × 4.3 mm	Cyclic	Developing
Non-castrate males	Developed. Avg 5.3 × 3.9 mm	No evidence of estrogenic stimulation*	Little or no development
Castrate males	Developed. Avg 8.0 × 7.0 mm	Non-cyclic cornified	Developing

* There may be a beginning mucinification of the epithelium suggesting estrogen-androgen cooperation.

castrate males by grafting bits of uteri and vaginae into the subcutaneous tissue of such mice. As a preliminary experiment, the uteri and vaginae of young F_1 hybrid females were removed, each uterine horn was split longitudinally, and each vagina was cut transversely into two narrow segments. Single uterine and vaginal fragments were then transplanted into the subcutaneous tissue (in either the axilla or groin) of several intact male and female F_1 hybrid mice. Two months later the recipient animals were sacrificed, and the grafts studied histologically.

The uterine grafts carried in female mice formed large thin-walled cysts lined with typical uterine epithelium. Because of the pressure exerted on the cyst wall by the accumulating fluid, the epithelial cells were generally rather low columnar or cuboidal in form. Only in areas of papillary infoldings were the characteristic tall columnar cells with significant amounts of underlying lamina propria found. In the intact male animals the uterine grafts had been reconstituted so as to form small cylindrical bodies very similar to the intact donor uteri before being split longitudinally. There was no tendency for fluid accumulation so that the normal uterine relationships were disturbed very little. Morphologically these grafts closely resembled uteri of castrate female mice except for a slightly "looser" appearance of the lamina propria.

Vaginal grafts were retained as cysts also. The epithelial lining seen in the grafts in the female animals faithfully reproduced that of the *in situ* vaginal epithelium, and the cyst lumen was filled with alternate layers of leukocytes and desquamated cornified epithelial cells. On the other hand, the epithelium of the vaginal grafts maintained in intact male mice consisted entirely of two layers of epithelium similar to that found in castrate female mice and the luminal contents were devoid of cellular components. This method of uterine and vaginal transplantation showed promise then of shedding light on the state of function of ovarian grafts carried in male animals.

Groups of 8 weanling hybrid mice were set up as follows: non-castrate males, castrate males, and ovariectomized females. Into each animal was transplanted 2 ovaries, a vaginal, and a uterine fragment. Two months later the animals were sacrificed, the uterine and vaginal grafts measured (2 diameters at right angles to one another), and histological preparations of all grafts studied. A summary of these findings is given in Table II. It is evident from the size and histology of the uterine grafts in the non-castrate male group that the ovaries of these animals were producing estrogenic substances in appreciable amounts, although in all probability, in lesser quantities than produced by the ovaries of the castrate group. In view of this, the morphology of the vaginal grafts is of considerable interest. The epithelium of the grafts in the non-castrate animals was formed throughout by only 2 layers of cells much the same as in the vaginal grafts of intact male mice without ovarian transplants. The only indication of development was a tendency for the luminal layer of cells to be low columnar in form and to possess an increased amount of clear cytoplasm. This appearance

TABLE III.
Development of Vaginal Grafts and Mammary Glands in Castrate and Non-castrate Male Mice After Injection of Various Doses of Estrone for 3 Weeks.

Amt of estrone		Vaginal mucosa	Mammary gland
.08 γ in 2 days twice per week	Castrate	Partial cornification with considerable mucinification	No development
	Non-castrate	No evidence of estrogen activity	No development
.05 γ per day	Castrate	Cornification with some areas of mucinification	Only 1 of 4 animals showed mammary development
	Non-castrate	Epithelial development mainly mucinification	Little or no development
.15 γ per day	Castrate	Completely cornified	Good mammary development
	Non-castrate	Cornification with some areas of mucinification	Little or no development

is suggestive of beginning mucinification which Korenchevsky and Hall[14] have shown to result from the cooperative action of estrogens and androgens or progesterone. The vaginal grafts in the castrate male animals are also worthy of some description, for although they appeared extensively stimulated, there was no indication of estrous cycling. The epithelial histology was the same in all 8 grafts; however, there was considerable variation from area to area within each graft. While most areas showed complete or almost complete cornification, others exhibited only partial cornification with leukocytes passing between the epithelial elements. The lumina were filled uniformly with desquamated cornified epithelium mixed with some leukocytes, but with no indication of stratification as was seen in the grafts carried in normal female mice. This "mixed" vaginal histology is similar to that seen in female mice subjected to a constant stimulation with estrogen.

Although the difference in uterine and vaginal-mammary development in the non-castrate male mice might reflect only a difference in tissue sensitivity to estrogen, it might also reflect an antagonism between the two classes of sex hormones with regard to the vaginal and mammary epithelium. This latter possibility was investigated by transplanting a group of hybrid male mice with uterine and vaginal fragments and allowing 2 weeks to elapse for vascularization of the grafts. Half of the mice were then castrated, and groups of castrate and non-castrate animals were injected with graded doses of estrone with 4 castrate and 4 non-castrate animals receiving each dose level. Injections were begun the day following castration in order to minimize the possibility of an increased estrogen production by the adrenals of the castrate animals. The estrone was injected subcutaneously in aqueous solution, the daily dose being equally divided between a morning and an afternoon injection. Animals at the lowest dosage level received 0.4 γ a day on 2 successive days and for 4 days of each week. The other groups received 0.05 and 0.15 γ daily 7 days a week. Injections were continued for 3 weeks, and the animals were autopsied within 6 hours of their last injection. A summary of the results is given in Table III. The data on the uterine grafts are not included as measurements on such a small number in each group could not be considered conclusive. It can be said, however, that at all dosage levels the uterine grafts became cystic and that there was no consistent difference in size between the grafts of castrate and non-castrate animals receiving the same amount of estrone, although the

[14] Korenchevsky, V., and Hall K., *J. Path. and Bact.*, 1937, **45**, 681.

grafts of the animals receiving 0.15 γ of estrone generally exceeded those of the other 2 groups.

The study of the vaginal grafts and mammary glands suggest two major points. First, there is a considerable difference in the sensitivity threshold of these 2 tissues to estrone. Second, there would appear to be some antagonism between testicular hormone and estrone. To establish this latter conclusion with certainty, however, differences in adrenal function must be completely ruled out, although under the conditions of this experiment significant differences in this respect seem unlikely. Although the state of function of the pituitary does not appear to alter the vaginal response to estrogen,[15] it has been found that mammary gland growth is dependent upon a functional pituitary gland (for review see [16]). In order to be certain that the amounts of estrone injected had not caused a sufficiently severe hypophyseal suppression to inhibit mammary growth in the non-castrate males, the testes, ventral prostate, and seminal vesicles of each animal were studied. In all instances these organs were grossly and microscopically indistinguishable from those of normal intact males. Since, in our experience, doses of estrogens considerably lower than that required to cause "stunting" of the mammary gland produce easily demonstrable reduction in the production of pituitary gonadotropin, it would appear that in these estrone injected non-castrate mice pituitary suppression was not the factor responsible for the reduced response of the mammary glands.

The non-castrate males bearing ovarian grafts lived significantly longer than do virgin female mice of this hybrid cross (those lacking the milk agent so that no mammary cancer developed.[10]) It is, therefore, of interest to record that in 4 of these animals typical granulosa cell tumors developed in the area of the transplanted ovary (Fig. 4). These tumors developed when the mice were very old: 24.9, 25.4, 30.2, and 32.2 months of age. In none of these 4 animals was there evidence of high estrogen levels since the mammae were no more developed than in other animals of the group.

Since it has been suggested that an increased pituitary gonadotropin level is responsible for ovarian tumor formation in the Biskind-Biskind type of experiment,[17,18] a group of 35 F_1 hybrid males lacking the milk agent were castrated and transplanted with ovaries in the axillary position. The average life of these males was 24.6 months, but only two granulosa cell tumors were observed: at 19.3 and 23.3 months. Although these tumors developed somewhat earlier in life than did those in the non-castrate males, it is evident that no increase in the frequency of this tumor was brought about by castration. One of these tumors was transplanted to the subcutaneous tissue of other F_1 hybrid mice and was observed to grow rapidly in intact as well as in castrate males and in intact females. No evidence of estrogen production by these transplants could be found.

As there are no data available on the spontaneous occurrence of granulosa cell tumors of the ovary in normal female mice of this hybrid cross to use for comparative purposes, the ovaries transplanted to oöphorectomized females of this strain[11] were reviewed. For this comparison only mice living to be 700 days of age or older were included. There were 18 female animals bearing hybrid grafts and living to an average age of 26.0 months available for study. In these only one atypical ovary possessing an overgrowth of granulosa cells was encountered (age 28.7 months). This is to be compared with the non-castrate male group bearing ovarian grafts in which 21 lived beyond 700 days of age (average age of death 28.0 months) and in which 4 typical granulosa cell tumors developed. Because of the small number of tumors observed and the somewhat longer average life of the male group, no statement as to the relative frequency of this

15 Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

16 Huseby, R. A., Ball, Z. B., and Visscher, M. B., *Cancer Research*, 1945, **5**, 40.

17 Biskind, M. S., and Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 176.

18 Li, M. H., and Gardner, W. U., *Science*, 1947, **105**, 13.

type of tumor in the two groups seems justified.

It seems of value, also, to record the occurrence of ovarian tumors developing in the ovaries of the two parent strains when transplanted to ovariectomized F_1 hybrid females. There were 11 hybrid females bearing Z or C_3H ovaries living beyond 700 days of age, and in these one mixed ductular-granulosa cell tumor was found (age 24.2 months). In 13 hybrid females past 675 days of age and bearing strain A ovaries, one typical luteoma (23.5 months) and two granulosa cell tumors (22.5 and 25.4 months) were encountered.

Discussion. It has been previously recorded that castrate male guinea pigs bearing ovarian grafts exhibit a greater mammary gland development than do normal virgin females (for review see [2]). The present experiments with mice show that castrate males with ovarian grafts developed mammary cancers earlier than do normal virgin females of this hybrid cross, in fact the average age of tumor development approached that seen in similar breeding females. Although Smelser[2] found some indication of cyclic pituitary function in castrate, ovarian grafted male guinea pigs, our experience with vaginal grafts in castrate, ovarian grafted male mice suggests that the gonadotropin stimulation from these male pituitaries is at all times such that sufficient ovarian estrogen is produced to extensively cornify the vaginal epithelium. Although the largest part of the epithelium of these grafts was completely cornified, areas were encountered in all in which leukocytes could be seen passing through an incompletely cornified epithelium. This "mixed" picture is consistent with that seen in vaginae subjected to long periods of continuous estrogen stimulation.

The failure of the mammal to develop in the non-castrate males bearing ovarian grafts is in agreement with the observations of Smelser in guinea pigs. It is, however, at variance with those of Gardner[3] who employed intratesticular grafts in CBA mice. This may, of course, be a strain difference. However, Gardner's photomicrographs show considerable tubular damage in the testes due to the ovarian transplant. It seems possible that this might explain the difference since Smelser observed mammary development in cryptorchid guinea pigs bearing intranephric ovarian grafts in which the androgen production by the cryptorchid testes was adequate to maintain the secondary sex glands but in which tubular alterations were extensive because of the intra-abdominal position of the testes. Further experimentation is necessary to establish or reject such an explanation.

The results of the experiments reported here designed to test the function of the ovarian grafts in the non-castrate male animals would seem to indicate that significant amounts of estrogen were being produced, but that the quantities were smaller than those produced by the ovarian grafts carried in castrate males. Any estimation of relative amounts of estrogen production by the grafts in these two groups of animals seems unwarranted since the picture appears to be further complicated by antagonism between estrogen and androgen with regard to vaginal and mammary gland development in the non-castrate group.

An estrogen-androgen antagonism for both the vaginal and mammary epithelium seems fairly well established by the results of the last experiment reported here. At the lowest dose level of estrone, the vaginal grafts in the castrate males showed definite histological evidence of stimulation while in the non-castrate group no demonstrable epithelial development was seen. At the higher dose levels, the cooperative action of the two hormones as described by Korenchevsky and Hall[14] is also clearly demonstrated. Robson[19] has suggested such an antagonism, but since he used only smears to follow the vaginal response one does not know whether he observed true antagonism or the co-operative effects resulting in epithelial mucinification which would also produce a "negative" vaginal smear. Gardner[20] has also shown this antagonism for the mammary gland by injecting estradiol benzoate and testosterone propi-

[19] Robson, J. M., *J. Physiol.*, 1937, **90**, 15.

[20] Gardner, W. U., *Cancer Research*, 1946, **6**, 493.

onate concurrently. The possibility that the antagonism in the mammae is due to pituitary suppression resulting in mammary gland "stunting" seems to be ruled out in our experiment in which relatively small doses of estrogen and the androgen from functioning testes were employed.

Conclusion. Castrate male mice of the F_1 hybrid generation between the A and Z stocks bearing subcutaneous ovarian grafts developed mammary cancer more readily than do virgin female mice of this cross. Only 1 of 39 non-castrate males bearing ovarian grafts develop mammary carcinoma as little mammary development occurred. Additional experiments seemed to indicate that the ovaries in the non-castrate male mice were producing an appreciable amount of estrogen although measurably less than those carried in castrate males. There appeared, also, to be an antagonism between estrogens and androgens as far as stimulation of the vaginal and mammary epithelium is concerned. Granulosa cell tumors occasionally occurred in ovarian grafts of old male and female mice of this hybrid cross.

16708 P

The Effect of 11-Desoxycorticosterone Acetate Upon the Glycosuria of Partially Depancreatized Rats.

DWIGHT J. INGLE.

From the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

This study shows that large doses of 11-desoxycorticosterone acetate cause an exacerbation of the diabetes of partially depancreatized, force-fed rats.

Methods. Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets until they reached a weight of 300 g. Ten rats were depancreatized by the method of Ingle and Griffith.[1] After diabetes was established all of the animals were placed in metabolism cages and were fed a medium carbohydrate diet made according to Table I. All of the animals were force-fed by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P. M.). The technics and diets were modifications of those described by Reinecke, Ball and Samuels.[2] During the period of adaptation to force feeding, the amount of diet was increased gradually to prevent "food shock." A full feeding of 26 cc of diet per rat per day was reached on the 5th day.

TABLE I.
Medium Carbohydrate Diet.

Constituent	G
Cellu flour (Chicago Dietetic Supply)	120
Osborne & Mendel salt mixture	40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Vitamin K (2-methyl-1,4-naphthoquinone)	100 mg
Mazola oil	200
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200
Water to make total of	2000 cc

The animals were kept at a temperature of 74° to 78°F and a humidity of 30 to 35% of saturation. Twenty-four-hour samples of urine were collected at the same hour (8:00 to 8:30 A.M.) and were preserved with thymol. Urine glucose was determined by the method of Benedict.[3]

The 11-desoxycorticosterone acetate (Cortate, Schering) was made up in sesame oil at 5 mg per cc. It was given by subcutaneous in-

[1] Ingle, D. J., and Griffith, J. Q., Chapter 16, The Rat in Laboratory Investigation, J. B. Lippincott Co., Philadelphia, 1942.

[2] Reinecke, R. M., Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 44.

[3] Benedict, S. R., *J.A.M.A.*, 1911, **57**, 1193.